

IN VITRO INVESTIGATION OF FRESH JUICES OF RIPE ANANAS COMOSUS (PINEAPPLE), CARICA PAPAYA (PAWPAW) AND CITRULLUS VULGARIS (WATER MELON) FOR UTERINE CONTRACTILE PROPERTIES IN NON-PREGNANT RATS

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ABSTRACT

Isolated rat uteri were used to investigate the effects of fresh juices of *Ananas comosus* (Pineapple), *Carica papaya* (Pawpaw) and *Citrullus vulgaris* (Water melon) on the rat uterus. Each female rat primed with diethyl stilbesterol (1mg/kg) 24 hours and starved for 12 hours before experiment was euthanized by a blow on the head and exsanguinated. The lower abdominal region was opened and the two horns of the uterus were carefully isolated and transferred into a beaker containing De Jalon solution at 37°C and aerated. One of the horns was mounted in a 35ml organ bath also containing De Jalon solution at 37°C with adequate aeration. After equilibrating for 30 minutes the effects of Acetylcholine and fresh juices of *Ananas comosus*, *Carica papaya* and *Citrullus vulgaris* were tested separately and repeated in the presence of Atropine. Results obtained showed that *Ananas comosus* juice significantly ($P < 0.05$) induced contractions of the uterine smooth muscles in a dose dependent manner, just like Acetylcholine, a standard muscarinic agonist, while ripe *Carica papaya* and *Citrullus vulgaris* fresh juices showed no contractile effect and did not block the effect of administered Acetylcholine. However, the contractions established by *Ananas comosus* could not be blocked by Atropine. The inability of Atropine to block the contractile effects of *Ananas comosus* juice suggests non-involvement of muscarinic receptors but leaves histaminergic receptors, oxytocin receptors and calcium channel alteration as possible mechanisms. The consumption of pawpaw and water melon juices may be encouraged at all stages in pregnancy while that of pineapple should be discouraged except at full term when its utero-tonic property can augment the action of oxytocin in labor and placenta removal after delivery.

KEYWORDS: Acetylcholine, *Ananas comosus*, *Carica papaya*, *Citrullus vulgaris*, Receptors, Uterine, Utero-Tonic

INTRODUCTION

Uterine contractions at any stage of pregnancy usually leads to the forceful removal of uterine contents. At full term, the release of oxytocin by the posterior pituitary gland causes contractions of the uterine smooth muscles and usually marks the onset of labor (Prema and Sembulingam, 2009).

Today, the advancement of science has led to the discovery and development of exogenous natural and synthetic substances that can boost the activity of the endogenous oxytocin so as to limit the complications associated with prolonged labor. While some of the substances are synthetic, a whole lot are of plants origin. Utero-tonic plants are plants that stimulate uterine contraction and have been used since the ancient times to assist labor, remove retained placenta, treat postpartum bleeding and as an abortifacient (Salleh and Ahmad, 2013). Some of the plant products reported to be used

traditionally to promote uterine contractions are fresh juices of ripe *Ananas comosus*, *Carica papaya* and *Citrullus vulgaris* (Personal Conversation, Michael Okpara University of Agriculture, Umudike).

Ananas comosus is a tropical plant with edible fruit consisting of coalesced berries. It is cultivated from a crown cutting of the fruit, possibly flowering in 20-24 months and fruiting in the following 6 months. It is an herbaceous perennial which grows up to 3.3 – 4.9 feet high with short stem and tough leaves. Phytochemical screening of the fruit reveal the presence of saponin, glycoside, flavonoid and tannins and vitamins such as B₁, B₂, B₃, B₅, B₆, B₉ and C. Minerals such as calcium, magnesium, phosphorous, potassium, sodium and zinc have also been identified in the fruit (Ikeyi *et al*, 2013). The fruit juice aids digestion and has been used to treat delayed stomach emptying (*Gastroparesis*) (Frank, and Jackson, 2014).

Carica papaya is a woody herb, growing up to 10-12 feet and is relatively short lived. Numerous substances of health value including carbohydrates, fibers, vitamins, A, B, B₂, B₃, B₆, C, E and K and minerals such as Iron, Zinc, Magnesium, Calcium, Copper and Selenium have been identified in *Carica papaya* fruits. Kassidy (2012) reported that the fresh fruits are eaten to treat rheumatism.

Citrullus vulgaris fruit is a spherical summer fruit with a light green or dark color, containing red pulp dotted with black seeds. The fruit is reported to contain carbohydrate, fiber, protein, free fat and cholesterol with vitamins A, C, E, D niacin, thiamine B₆, B₁₂ and minerals such as Iron, Calcium, Magnesium, Manganese, Phosphorus, Zinc, Fluorine, Selenium (Abdulrazaq, (2012).

The roots, stems and leaves of these plants have been used for medicinal purposes with little emphasis on the potentials that abound in their fruits. Most times pregnant mothers are advised to eat a lot of the fruits without considering their possible systemic effects. This work is therefore designed to study the effects of fresh juices of Pineapple, pawpaw and water melon on the uterine smooth muscles of Rats with the aim of making useful recommendations on their consumption during pregnancy.

MATERIALS AND METHODS

Collection of Fresh *Ananas comosus* (Pineapple), *Carica papaya* (Paw Paw) and *Citrullus vulgaris* (Water Melon) and Extraction of Their Juices

Freshly ripe pineapple, pawpaw and water melon fruits were bought from local fruit dealers in Ahiaeke fruit market, Umuahia North Local Government Area of Abia State. The fruits were washed and the epicarp peeled off to expose the succulent mesocarp. A manual juice extractor was used to extract fresh juices from each of the fruits. The juices were kept in a refrigerator until needed.

Animals

Ten adult female Rats (160-230g) obtained from the Animal production unit of the Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health, Michael Okpara University of Agriculture, Umudike were used for the study. The rats were feed with standard feed (vital feeds, Nigeria) with water ad libitum and housed in an aluminum cage. Each rat was primed with subcutaneous injection of Stilbestrol (1mg/kg) 24 hours and starved 12 hours before experiment. All animal experiments were conducted in compliance with NIH guidelines for care and use of Laboratory Animals (Pub. NO. 85-23, Revised 1985) as expressed by Akah *et al.*, (2009). Experiments for this study were

carried out in the physiology laboratory of the Department of Physiology, Pharmacology, Biochemistry and Animal Health, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation and Dilution of De Jalon Solution and Standard Drugs

De Jalon solution, the physiological salt solution most suitable for isolated rat uterine preparations was prepared such that each liter of water contained NaCl - 9g, KCl-0.42g, CaCl₂-0.06g, NaHCO₃- 0.59 and Glucose - 0.5g (Anaga *et al.*, 2010). 1 x 10⁻¹g/ml of standard drugs (Acetylcholine and Atropine) was prepared (w/v) by carefully weighing 0.5g of the drugs and dissolving same in 5ml of distilled water. Direct dilutions were subsequently made from these stock concentrations to obtain 1 x 10⁻⁶g/ml of the drugs.

Final Bath Concentrations (FBC) was Calculated Using the Formula:

$$FBC = \frac{C_1 V_1}{V_2}$$

V₂

Where

C₁ = Initial drug concentration

V₁ = Initial drug volume

V₂ = Final volume (volume of organ bath)

Fresh juices of *Ananas comosus*, *Carica papaya* and *Citrullus vulgaris* were used in the concentrations in which they were obtained but expressed as percentages of the volume of the organ bath. Percentages of administered fruit juices were obtained using the expression

$$\% = \frac{\text{Volume of juice administered}}{\text{Volume of Organ bath}} \times 100$$

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In vitro Effect of *Ananas comosus*, *Carica papaya* and *Citrullus vulgaris* on the Rat Uterus

A primed and fasted rat was euthanized by a blow in the head followed by exsanguinations. The lower abdominal/pelvic region was opened and the two horns of the uterus carefully traced, isolated and transferred into a beaker containing De Jalon solution at 37°C and aerated. One of the horns was trimmed of fatty tissue attachments and was mounted in a 35ml organ bath containing De Jalon solution at 37°C and well aerated. The mounted tissue was allowed to equilibrate for 30 minute, after which dose response relationships were established using the following drugs.

- Acetylcholine Alone
- *Ananas comosus* juice alone
- *Carica papaya* juice alone
- *Citrullus vulgaris* juice alone
- Acetylcholine in the presence of Atropine
- *Ananas comosus* juice in the presence of Atropine

- Vii. Acetylcholine in the presence of *Carica papaya* and *Citrullus vulgaris*

The entire procedure was repeated with the remaining 9 Rats on different occasions.

Statistical Analysis

Results were expressed as means \pm Standard Error of Mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA). Student's t-test at 95% level of significance was used to assess significant differences between uterine activities in basal and treated states. P values less than 0.05 were adjudged significant.

RESULTS

Effects of Acetylcholine and Fresh Juices of *A. comosus*, *C. papaya* and *C. vulgaris* on Rat Uterus

The isolated rat uterus did not exhibit the usual spontaneous wavy contractions typical of such preparations, hence the baseline or basal contractions stood at 0.00 mm. Acetylcholine significantly ($P < 0.05$) induced contractions of the isolated uterus with the highest amplitude of 18.10 ± 0.08 mm obtained at an FBC of 2.29×10^{-8} g/ml. The fresh juice of *Ananas comosus* also significantly ($p < 0.05$) induced contraction of the isolated uterine tissue with a 2.29% concentration causing a response of 15.60 ± 0.13 mm. The effects of *A. comosus* juice was not significantly ($p > 0.05$) different from that of Acetylcholine. The fresh juices of ripe *C. papaya* and *C. vulgaris* showed no contractile effect on the isolated rat uterus.

Effect of Atropine on Acetylcholine and *Ananas comosus* Juice Induced Uterine Contractions

Atropine (1.43×10^{-9} g/ml) significantly ($p < 0.05$) blocked Acetylcholine induced uterine contractions but had no significant effect on the contractions induced by *Ananas comosus* fresh juice. Fresh juices of ripe *Carica papaya* and *Citrullus vulgaris* fruits which did not induce uterine contractions in the isolated tissue did not also block the effect of Acetylcholine.

Table 1: Effects of Acetylcholine, and Fresh Juices of *Ananas comosus*, *Carica papaya*, and *Citrullus vulgaris* on the Rat Uterus

FBC of Acetylcholine (g/ml)	V/V Concentration of Juices (%)	Amplitude Acetylcholine (mm)	Amplitude <i>A. comosus</i> (mm)	Amplitude <i>C. papaya</i> (mm)	Amplitude <i>C. vulgaris</i> (mm)
2.86×10^{-9}	0.29	5.20 ± 0.07	5.30 ± 0.009	0.00 ± 0.00	0.00 ± 0.00
5.71×10^{-9}	0.57	11.00 ± 0.08	8.90 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
1.14×10^{-8}	1.74	15.10 ± 0.14	12.20 ± 0.11	0.00 ± 0.00	0.00 ± 0.00
1.7×10^{-8}	1.71	15.10 ± 0.14	12.20 ± 0.11	0.00 ± 0.00	0.00 ± 0.00
2.29×10^{-8}	2.29	18.10 ± 0.08	15.60 ± 0.13	0.00 ± 0.00	0.00 ± 0.00

$P < 0.05$ when compared to basal values of 0.00 mm

Table 2: Effects on Rat Uterus of Acetylcholine and *Ananas Cosmosus* Fresh Juice in the Presence of Atropine (1 X 43 X 10^{-9} g/ml)

FBC of Acetylcholine (g/ml)	Concentration of <i>Ananas cosmosus</i> Juices (%)	Amplitude Acetylcholine + Atropine (mm)	Amplitude <i>A. comosus</i> + Atropine (mm)	Acetylcholine + <i>C. papaya</i> (mm)	Acetylcholine + <i>C. vulgaris</i> (mm)
2.86×10^{-9}	0.29	2.7 ± 0.09	5.9 ± 0.05	6.20 ± 0.00	5.80 ± 0.00
5.71×10^{-9}	0.57	5.80 ± 0.10	7.80 ± 0.08	11.30 ± 0.08	12.00 ± 0.08
1.14×10^{-8}	1.14	6.10 ± 0.08	10.00 ± 0.09	12.10 ± 0.07	12.70 ± 0.00
1.7×10^{-8}	1.71	7.20 ± 0.11	11.40 ± 0.09	14.00 ± 0.09	13.20 ± 0.09
2.29×10^{-8}	2.29	8.0 ± 0.10	14.70 ± 0.13	17.50 ± 0.11	18.10 ± 0.09

$P < 0.05$ for *A. comosus* + Atropine when compared to basal 0.00 mm value

DISCUSSIONS

Acetylcholine, a muscarinic receptor agonist induced contractions of the isolated rat uterus evidenced by the dose dependent increase in Amplitude of contractions. Acetylcholine achieved this effect by the binding of the drug molecules to the muscarinic receptors present in the smooth muscles of the uterus. Available literature reveals the presence of numerous muscarinic receptors particularly the M₂ and M₃ types in the uterine smooth muscles (Choppin *et al.*, 1999; Pennefather *et al.*, 1994 and Kitazawa *et al.*, 2007). An important result of muscarinic agonist binding is the activation of the inositol triphosphate (IP₃) and diacylglycerol (DAG) cascade. Some evidence implicates DAG in the opening of the smooth muscle calcium channels. IP₃ evokes release of calcium from the endoplasmic and sarcoplasmic reticulum. Muscarinic agonist also increase cellular concentration of cyclic Guanine Monophosphate(GMP) and activation of muscarinic receptors increases potassium flux across cell membrane (Bafar and Sanni, 2009). This chain of physiological processes mediates uterine contractions.

The fresh juice of ripe *Ananas comosus* when administered to the isolated uterine tissue induced contractions in a manner similar to that of Acetylcholine while fresh juices of ripe *Carica papaya* and *Citrullus vulgaris* showed no contractile effect on the tissue. Atropine, a muscarinic receptor blocker significantly blocked the effects of administered Acetylcholine but could not block the contractile effects of *A. comosus* juice which suggest that *A. comosus* induced uterine contractions may not have been mediated via muscarinic receptors. The detailed mechanism through which *Ananas comosus* juice induces uterine contraction remains unclear and an area for further studies but may be through binding to Histaminergic (H₂) receptors present in rat uterus (Aucellio, 1988), promoting calcium flux in the smooth muscles via the uterine L-type calcium channel (Collins *et al.*, 2000), binding to oxytocin receptors in the uterus (Alexandrova and Soloff (1979), and the effect of bromelain, an enzyme present in *Ananas comosus* juice which has been implicated in the induction of uterine contractions (Treato.Com 2014, Personal conversation, Michael Okpara University of Agriculture, Umudike, 2014).

CONCLUSIONS

From the results obtained it becomes reasonable to conclude that although the fresh juice of *Ananas comosus* is enriched with vitamins, minerals and other health promoting substances, its consumption before term in pregnancy should be discouraged to prevent premature induction of uterine contractions which may lead to abortions or miscarriages. Its consumption may however be encouraged at full term and onset of labor, since as an utero-tonic agent, it contains active principles which can enhance oxytocin induced uterine contractions, assist labor and help remove retained placenta. The consumption of fresh juices of ripe *Carica papaya* and *Citrullus vulgaris* may be encouraged at all stages of pregnancy since they have been found to possess magnificent health benefits with no utero-tonic property. The result of this work tend to agree with the belief on uterine contractile property of *Ananas comosus* but fail to agree with the claim that the consumption of ripe *Carica papaya* and *Citrullus vulgaris* fruits juices can induce uterine contractions.

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